

**Contributions to the Study of Pathogenic  
Amœbæ from Bombay.**

By

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and

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With Plates 16-18.

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**Part I.—An Examination of Some Cultures of Amœbæ isolated  
from Dysenteric Lesions and Other Sources. By W. Glen  
Liston.**

ANYONE who has attempted to study the literature on amœbic dysentery and liver abscess must have been struck by the confusion existing at present as to the exact differentiation and life-history of the amœbæ, which have been reported as the causative agents of this disease. This confusion can be accounted for, in large measure, by the inadequate and incomplete study of the morphology and development of the amœbæ which have been supposed to be the causative agents.

Schaudinn (3) first warned us that all amœbæ to be found in the human intestine were not of the same species. Using the method of building up a life-history from the study of the changes which are observed in a series of individuals at different stages of development—a method which, in his hands, had yielded in many instances splendid results—Schaudinn showed that there were at least two species of amœbæ to be found in the human intestine: the one non-pathogenic, which he named *Entamœba coli*, the other

pathogenic, which he named *Entamœba histolytica*. A number of other workers using his methods have discovered still other species, viz. *E. tetragena*, *E. minuta*, etc. More recently Musgrave and Clegg (1) in the Philippine Islands, and Noc (2) in French Cochin China, have stated that they have isolated by culture from liver-abscesses and dysenteric stools a somewhat polymorphic amœba, which they regard as the causative agent of liver-abscess and dysentery in their respective countries. They have also found the same species in a number of other situations—in drinking water, on vegetables, and in the intestines of healthy men and animals. In fact, they seem to think that all amœbæ which can be cultivated on their agar medium, and which form cysts varying in diameter between 7 and 16 $\mu$ , belong to one species,<sup>1</sup> a species which can at one time pass a harmless existence outside the body, at another time, when conditions are favourable, invade the tissues, and give rise to the grave lesions associated with dysentery.

The present paper has been written to show that at least two very distinct species of amœbæ have been found in cultures obtained after the manner of Musgrave and Clegg. I am, however, not yet in a position to state whether both or either of these species are really the causative agents of dysentery and liver-abscess. I am indebted to Captain Wells, of the Indian Medical Service, who has for some time been

<sup>1</sup> While Musgrave and Clegg in their monograph on "Amœbas" (Bureau of Govt. Laboratories, No. 18, p. 77), state that "The cultivation of pure species of amœbas has offered strong evidence of the plurality of the species of these protozoa, and this plurality apparently extends to those which produce infection in man." in a later publication Woolley and Musgrave, in a paper published in the same series, No. 32, June, 1905, write: "It may be well to state at the outset that we can see no valid reason for departing from the nomenclature of Lösch. He described a pathogenic amœba and called it *Amœba coli*. Why this term should be applied to a supposititious non-pathogenic organism it is difficult to say. We shall, in referring to the cause of intestinal amœbiasis, use the name introduced by Lösch." The measurements of the cysts are those given by Noc.

engaged in the study of dysentery in India, for the cultures I have used. These cultures of amœbæ I understand were obtained in Bombay from the following sources: (A) a liver-abscess; (B) a dysenteric stool; (C) Bombay City tap-water; (D) the stool of a healthy monkey.

A period of leave to England presented me with a favourable opportunity for studying these cultures under the control and guidance of a skilled protozoologist. I have to thank Dr. Martin, the Director of the Lister Institute, and Professor Minchin, of the London University, for allowing me to work in the laboratory at the Lister Institute, and the latter especially for drawing up for me a scheme of research in connection with the cultures I had obtained, and for instructing me in the best methods for studying the amœbæ.

Professor Minchin suggested that I should endeavour to ascertain whether the amœbæ from these different sources were one and the same species, by studying their morphology and development in cultures, and, by using the physiological test, attempt to produce dysenteric lesions in animals. He further suggested that I might find out whether these parasitic amœbæ absorb food osmotically, like a trypanosome, or must devour things like the ordinary free-living amœbæ. I was to endeavour, if possible, to get pure cultures free from bacteria.

A preliminary examination of the cultures, which had been planted more than a month previously, showed that in each case all the amœbæ were encysted, and that in all they were associated with a variety of bacteria. The size of the cysts in the different cultures varied considerably, but it was observed that on the whole the cysts in the culture obtained from a dysentery-stool were smaller than those found in the cultures obtained from Bombay tap-water and from the monkey. The cysts in the liver-abscess culture varied more considerably than the other cultures; many very small cysts, often grouped together, were mixed with cysts as large as those found in the water or the monkey culture. It appeared, then, that in the case of the liver-abscess culture two types of cysts were present. Measurements of the cysts showed

that while the smaller type varied in diameter between 6 and 8  $\mu$ , the larger types varied in diameter between 12 and 15  $\mu$ . To determine whether the amœbæ which developed from these cysts remained true to type, a single cyst was isolated under a lower power of the microscope on the point of a brush or needle which had been dipped in gum, and the cyst was then transferred to fresh culture-medium. It was found that the amœbæ always remained true to type, that is to say, large cysts were always formed by amœbæ which had developed from a single large cyst, while small cysts were always the product of small cysts. Moreover, the amœbæ from the large cysts were considerably larger than the amœbæ from the smaller cysts. There thus appeared at first sight to be two species of amœbæ present in the liver-abscess culture. This supposition was further substantiated by the fact that the other two types differed in respect to (A) their methods of multiplication; (B) their behaviour in saline solution; and (C) in other minor points. These points of difference will now be discussed.

#### (A) METHODS OF MULTIPLICATION ON CULTURE-MEDIUM.

The large amœba was observed to multiply by endogenous budding as well as by division into two individuals. On one occasion a living amœba in a pure culture derived from large cysts was watched for some hours. During this period three or four buds were formed within the body of the amœba and liberated from it. This method of multiplication was more easily followed in fixed and stained specimens. Budding was observed both in old and in young cultures. In one case, an amœba, which from its size appeared only lately to have emerged from its cyst, already contained a bud almost as large as itself (Pl. 17, fig. 21), while an older and larger amœba showed as many as six buds in various stages of development (Pl. 16, fig. 4). It will be observed that the method of budding here demonstrated (Pl. 16, figs. 1-5, Pl. 17, figs. 16-20) differs materially from that described

and figured by Noc (2), who describes the buds he observed as very small, each bud containing a single minute fragment of chromatin. So numerous were the buds within the body of the parent amœba that he says the term "merozoite" could be aptly applied to the bred. In my experience great difficulty was found in differentiating between the bodies of bacteria ingested by an amœba and lying within its cytoplasm and small fragments of chromatin or chromidia. It must be remembered that in cultures the amœbæ are always present with vast numbers of bacteria. Although a number of stains were used none was found which satisfactorily distinguished bacteria from chromidia. It was therefore difficult to trace the origin of the buds. The buds became recognisable within the cytoplasm of the parent amœba, when a larger mass of chromatic material was assembled than could be reasonably explained on the supposition that it was formed from ingested bacteria. Around these collections of chromatic material the cytoplasm of the amœba becomes differentiated from the rest of the cytoplasm of the body. As this differentiation becomes more and more marked the enclosed chromatic material more and more closely resembled the structure of a typical nucleus, till finally, within the body of the amœba, a completely differentiated amœbula is developed. At this stage the amœbula escapes from the parent amœba, and can then live a free existence, grow and develop into a full-sized adult amœba.

Division of this large amœba from the liver-abscess culture into two individuals was also observed in the living condition. On one occasion a single amœba was watched continuously for two hours and fifty-four minutes, and a detailed record was kept of the various events that occurred during this period. The culture in which this amœba was observed had been planted five and three-quarter hours before the observations commenced. A number of amœbæ had already escaped from their cysts while still others had not yet emerged from their cysts. The amœba which was selected for observation was a very active one. In progressing over

the culture-medium it made its way by extruding two distinct types of pseudopodia, viz. (1) coarse lobose pseudopodia, consisting almost entirely of ectoplasm, were extruded for the most part in the direction of movement, and (2) fine, needle-like, short, pointed pseudopodia extended out from the lobose pseudopodia as well as from other parts of the body. A nucleus and contractile vacuole were plainly visible. The vacuole contracted rhythmically and fairly regularly almost every two minutes. The amœba moved over the medium at about the rate of  $160\mu$  in thirteen minutes. As it progressed it encountered in its path little groups of cocci which were growing with it on the culture-medium. During one hour and thirty-eight minutes before the amœba divided into two individuals, one hundred and eight cocci were ingested by the amœba under observation. The cocci were taken into the protoplasm of the amœba in groups of two or four. When larger groups of cocci were encountered the amœba broke them up into smaller groups and then ingested them. Four minutes after ingestion the cocci were seen to be contained within a vacuole. The vacuoles containing the cocci moved about through different parts of the protoplasm, and as digestion proceeded they at first became larger, then, becoming smaller, they slowly disappeared. (On a neutral-red medium it was easy to demonstrate that the contents of the vacuoles had an acid reaction.) The cocci within the vacuoles for a time remained visible, but gradually, in the course of fifteen minutes, melted from view. After watching the amœba for one hour and thirty-eight minutes, it gradually became more and more sluggish and the contractile vacuole contracted less and less frequently. These changes occurred immediately after the amœba had engulfed piecemeal a group of thirty cocci, the amœba appearing to be satiated with this big meal. The amœba had meanwhile assumed a rounded form, withdrawing all lobose pseudopodia, but still the fine needle-like pseudopodia projected from its surface. The movements of the protoplasm, which up to this time had been active, gradually ceased altogether, and

the protoplasm became more and more granular. The nucleus, which up to this point had been clearly visible, could now no longer be distinctly defined. No movement of any sort was noticed for a few minutes. Some ten minutes after the amœba had engulfed the last cocci, and a few minutes after all movement had ceased, the amœba slowly changed from a rounded to a more oval form, becoming oblong, then elongated. Two small indentures next appeared in the protoplasm on either side, about the middle of the body. These indentures deepened, till, meeting, two amœbæ were formed. Division was complete in three minutes after the first appearance of the indentures mentioned above, and about thirteen minutes after the last cocci had been taken up as food. No trace of a nucleus could be made out in either of the two new amœbæ immediately after division. The newly formed amœbæ soon moved away from one another, and in three minutes one of them had approached and engulfed a group of nine cocci. A nucleus now gradually appeared in each amœba, but not till five minutes after division had been completed could it be clearly defined, meanwhile, as has been remarked above, the amœbæ moved about and even fed. Each amœba by this time had a contractile vacuole which rhythmically contracted about once every two minutes. The young amœbæ were watched for nearly an hour longer; no fresh points of interest, however, were noticed. The observations were abruptly terminated by an accidental knock to the Petri dish, which caused the amœbæ under observation to be moved out of the field of vision, and made it impossible to recognise with certainty those which had been watched from other amœbæ in the culture. I have not been able successfully to follow in a series of stained specimens the various changes described above seen in the living amœbæ. (Since writing this, with the assistance of Mr. C. H. Martin, divisional forms have been studied in stained specimens. The division is indirect or by mitosis.)

While thus the large amœba from the liver-abscess culture

showed two methods of multiplication, the small amœba, cultivated from the small cysts of the same culture, was never seen to give off buds. Actual division in the living state was not observed in the case of this amœba, but in stained preparations dividing forms were fairly easily found. Division in this case is direct or amitotic. Three such forms have been sketched from a single slide, while in other slides other stages of division have been observed.

(B) THE BEHAVIOUR OF THE AMŒBA IN SALINE SOLUTION.

One of the most striking differences between the two species of amœbæ found in the liver-abscess culture was brought into evidence when dilute saline solution 5 per cent. was added to an actively developing culture, time being allowed for the amœbæ to recover from the shock produced by the addition of the liquid. To demonstrate this difference between the two species of amœbæ the best results were obtained by placing a drop of the mixture of the amœbæ and saline solution on a slide and allowing it to remain for a few minutes in a warm moist chamber before fixing in Flemming's solution or sublimate-acetic mixture. The drop containing the amœbæ must not be covered with a cover-glass, but should be spread out in a thin layer and left freely exposed in a moist chamber. Adopting this method of preparing and fixing specimens, it was found that the large amœba always assumed a more or less rounded form, with lobose and numerous fine needle-like pseudopodia projecting from the surface. The small amœba on the contrary almost always assumed an elongated worm-like or gregarine shape, throwing out one or two long lobose pseudopodia either directly in front or often from the side so that L-shapes and Y-shapes, as well as long I-forms, were developed.

That Noc was dealing with both these types of amœbæ is evident, not only from the measurements of the cysts he worked with, but also from the drawings on his plate X. The drawings show both forms, the majority representing the



large amœba type, while his fig. 20 is a typical specimen of the small amœba described above.

(c) MINOR POINTS OF DIFFERENCE.

Other less important differences were noted between the two types of amœba found in the liver-abscess culture. Thus, for example, the nucleus of the large amœba in the living state was more clearly seen than that of the small amœba. The ectoplasm, too, was more clearly differentiated from the endoplasm in the case of the large amœba than in the small amœba. Then, again, a yellow pigment-producing coccus, which was a peculiarly favourable organism to grow with the large amœba, appeared to be quite unsuitable for the smaller amœba.

From what has been said above, it is evident that two very distinct species of amœba were present in this liver-abscess culture, and although a somewhat limited amount of attention has been given to the cultures derived from other sources, it is possible that the cultures from the Bombay City water and from the monkey, which contained an amœba very closely resembling the large amœba from the liver-abscess culture, nevertheless differed from it. The small amœba from the liver-abscess culture very closely resembled that found in the culture from a dysenteric stool.

An attempt was made further to differentiate between the two species of amœbæ found in the culture from liver-abscess by carrying out experiments on animals. For the purpose of these experiments young kittens were selected. The two species of amœbæ were isolated in pure mixed culture, and, when the amœbæ had encysted, an emulsion of each species was made in saline solution. Four kittens, whose fæces in the first instances had been examined microscopically and by culture for the presence of amœbæ with negative results, were experimented upon as follows: Two were fed by means of a small stomach-tube, the one with an emulsion of large amœba-cysts, the other with an emulsion

of small amœba-cysts. The two other kittens were injected per rectum, the one with an emulsion of large amœba-cysts, the other with an emulsion of small amœba-cysts. On the following day, as well as on the third day after treatment, the stools were examined both microscopically and by cultures for the presence of living amœbæ, but none were found. The kittens were healthy a fortnight later. These physiological tests were, then, unsuccessful in demonstrating any pathogenic properties in either species of amœba found in the liver-abscess culture, and they also failed to distinguish the one species from the other. Too much stress, however, cannot be placed on the failure of these experiments, not only because of their small number, but especially when the complicated conditions which are associated with the living together of bacteria and amœbæ are kept in mind, and to which some reference will be made later. Nevertheless, the negative results of these experiments are in conformity with the more numerous ones carried out by Noc and reported by him (2). By way of contrast, I found it interesting to study sections of the large intestine of a cat which had been infected with dysentery by the rectal injection of the stools of a patient suffering from dysentery in the Straits Settlements. This material was kindly supplied to me by Dr. Ledingham. Sections of this tissue showed well-marked dysenteric lesions associated with the presence of amœbæ, the amœbæ being found together with bacteria, not only in the mucosa, but penetrating into the submucosa in the neighbourhood of the ulcerations. It is interesting to compare the morphological appearance of these amœbæ with those found in the cultures. In the first place, the amœbæ in the sections of the cat's intestine were considerably larger than even the larger type found in my cultures. The nuclear structure of these amœbæ, too, differed remarkably from that of the amœbæ of the cultures. Thus, while the chromatic substance of the nucleus of the cultural amœbæ was abundant and differentiated into a large central portion and a thin peripheral layer, the chromatic substance of the

nucleus of the amœbæ found in the sections was very scanty, and confined for the most part to the periphery of the nucleus. These observations are in accord with those of Noc, who, when writing of the amœbæ which he isolated in culture and comparing them with Schaudinn's *E. histolytica*, and living forms isolated from the walls of a liver-abscess, points out that the cultural amœbæ were always smaller and contained more chromatin in the nucleus than those found in the tissues of a liver-abscess or of a dysenteric ulcer. How far these differences can be accounted for by differences in the methods of fixation and by the nature of the medium in which the amœbæ have respectively developed it is difficult to say, yet it is evident that the amœbæ which grow in cultures differ markedly in morphological characters from those found in sections of diseased intestine. Further study with fresh pathological material seems to be necessary before any definite conclusion can be arrived at as to whether the amœbæ obtained in cultures are the true cause of dysentery.

A few more details of the development of the amœbæ in cultures remain to be mentioned. The following observations concern particularly the large amœbæ of the liver-abscess, which was studied in greater detail than the smaller amœbæ. A single amœba has frequently been watched as it became encysted. Coming to rest the amœba gradually shrinks and becomes condensed. During this period the contractile vacuole beats more and more slowly; finally, having attained often an unusually large size, it very slowly shrinks and disappears. Other vacuoles in the protoplasm also disappear. Then a cyst-wall develops around the amœba. A single nucleus is alone visible within the cyst as long as it remains transparent and its contents can be stained.

When cysts formed in this way are planted on fresh medium the first change that is noticed is a gradual swelling of the cyst and the development of an increasingly large vacuole within it. The cyst-wall soon ruptures at some indefinite point and a single amœba slowly flows out through the breach.

It is apparent that when conditions are unsuitable for the growth of the amœba, a single individual by condensation of its protoplasm and the development of a cyst-wall becomes encysted, and, when the conditions are favourable, a single amœba escapes from this cyst. No evidence of conjugation was observed before encystment and no multiplication or division of the nucleus noticed, either immediately before or after encystment. Encystation appeared to be a purely protective measure. Outside the body the amœba was able to feed and to multiply under favourable circumstances, but it encysted when circumstances were unfavourable for development.

I will pass on now to describe some experiments which were made with a view to finding out whether the large amœba of the liver-abscess culture could absorb food osmotically and live and multiply on the agar medium in the absence of bacteria.

Much time was spent in the first instance in attempting to get the amœbæ from the four sources mentioned above to grow with a single species of bacteria. This was comparatively easily accomplished with the large amœba from the liver-abscess culture, but with greater difficulty in the case of the other amœbæ. All the "pure mixed" cultures were ultimately obtained with motile organisms except in the case of the large amœba from the liver-abscess. In this case the amœba was cultivated with a variety of motile and non-motile organisms, but particularly with a non-motile, yellow, pigment-producing coccus. With this pure mixed culture a number of experiments were conducted, and these are detailed below.

This amœba with the non-motile coccus multiplied enormously on the agar medium. The young amœbæ moved away from the immediate neighbourhood of the bacteria and ultimately became encysted. Using such a culture it was comparatively easy to separate amœba cysts from bacteria. With these cysts free from bacteria a number of experiments were carried out.

(1) A single cyst, free from bacteria, was isolated and

placed on fresh agar medium. A single amœba twelve hours later was found to have escaped from the cyst and was moving over the medium. Forty-eight hours later a single amœba was alone still actively moving over the culture medium; no multiplication had occurred. On the third day the amœba had become encysted again. In this case a single amœba had emerged from a single cyst and moved about for two days on the culture medium, which was free from any bacterial growth; no multiplication had taken place, but on the third day encystation had again occurred.

(2) A few cysts free from bacteria were planted out on an agar medium which, in the first place, had been smeared on one occasion with fresh sterile mouse's liver, on another occasion with fresh sterile unheated guinea-pig's liver. The amœbæ in due course emerged from their cysts and were observed to feed upon the red blood-cells and broken-up liver-cells of the smeared agar surface. The liver-cells which were not damaged or broken were too large to be engulfed by the amœbæ, which were approximately of the same size as these cells, some amœbæ, however, made attempts to swallow whole liver-cells, spreading themselves around the cells in a thin layer, but never succeeding in completely surrounding the liver-cell. A single amœba on one occasion was watched for two hours and a half while moving about on the agar medium smeared with fresh sterile guinea-pig's liver. During this time this amœba ingested and digested four red blood-cells, and attempted as well to take up a number of liver-cells as large as itself.

While thus the large amœba of the liver-abscess culture was seen to feed upon red blood-cells and broken down liver-cells, very little multiplication of the amœbæ was noted. Generally, without dividing, the amœbæ became encysted again. This was inferred from the fact that the number of cysts found after some days was very little in excess of the number originally placed on the medium. With the object of stimulating multiplication of the amœbæ the following procedure was adopted:

A number of sterile agar plates were smeared with fresh sterile guinea-pig's liver. These were placed in the incubator for two days to make sure that no bacteria had been planted on the surface of the medium during the preparation of the plates. Then to plate (A) a drop or two of an emulsion of the yellow coccus in saline solution, which had been boiled, was added at the same time as some bacteria-free cysts were planted on the liver-smeared culture. To (B) a drop or two of sterile saline solution was added as well as a few bacteria-free cysts. To (C) a drop or two of very weak sterile sodium carbonate solution with bacteria-free cysts was added. To (D) a few living yellow cocci in salt-solution were added with bacteria-free amœba-cysts.

The plates were then placed in the incubator and on the following day were examined. In each case living and moving amœbæ were observed. The plates were kept under observation for some days longer, and it was noted that while very little multiplication of the amœbæ had occurred in cultures A, B, and C in D, in the neighbourhood of the colonies of cocci, the amœbæ had multiplied enormously.

So far as it is possible to judge from a single experiment like the above, it would appear that multiplication of the amœbæ only occurs in the presence of some substance which is apparently connected with the life of the bacteria, a substance which is destroyed by boiling. In this connection the following casual observations are worthy of record: On a number of occasions it was noticed that if the number of cocci planted with the amœba-cysts was considerably in excess of the number of cysts, and particularly if the medium in which the culture was made favoured the development of the cocci, the amœbæ failed to develop, while the cocci flourished. On two or three occasions, when apparently a culture of amœbæ was growing well and multiplying in the presence of a colony of the yellow coccus, a stage was reached when the bacteria appeared to have produced some secretion which, diffusing outwards from the colony, caused those amœbæ which were in the immediate neighbourhood of the

colony, and which had not become encysted, to be dissolved, the amœbæ appearing to break up, melt, and disappear. On one occasion this condition was brought about by stirring up and spreading a little of the yellow coccus colony which was growing in the midst of a luxuriant culture of amœbæ.

These observations lead me to believe that a very delicate balance is maintained in a successful culture of amœbæ and bacteria. While on the one hand the amœbæ eat up and digest the bacteria, the bacteria, on the other hand, seem to produce some substance or substances which at one time stimulates the amœbæ to multiply, at another time actually brings about their destruction.

From what has been said above it is evident that I have not succeeded in showing that this amœba can absorb its food osmotically or live successfully without living bacteria; but I think certain lines for future research have been opened up which may ultimately explain the necessary connection which seems at present to exist between bacteria and the development and multiplication of amœbæ outside the body.

No one can be more conscious of the incompleteness of the work detailed above than myself. This paper has been written only because an opportunity may not present itself in the immediate future to continue this inquiry on my return to duty in India. It seems to me important, in the presence of a number of different species of amœbæ which may be found inhabiting the human intestine or in dysenteric lesions, that greater care should be taken in distinguishing one species from another, and specially the pathogenic from harmless commensal amœbæ.

#### CONCLUSIONS.

Two distinct species of amœbæ isolated from a liver-abscess have been cultivated on an agar medium. One at least of these amœbæ in cultures does not multiply in the absence of living bacteria. The same bacteria which, when

alive, stimulate the amœbæ to multiplication, when boiled and eaten by the amœbæ lack this power.

Amœbæ have been seen to feed upon and digest red blood-corpuscles, but in the absence of living bacteria failed to multiply on agar cultures.

LISTER INSTITUTE.  
November, 1910.

## **Part II.—Descriptions of Preparations of Amœbæ from Major Liston's Cultures. By C. H. Martin.**

In November of last year, Major Liston was kind enough to hand over to me some preparations and cultures of amœbæ, with a request that I should look through them. The preparations were made from cultures of five different strains of amœbæ, under the circumstances which Major Liston has described in Part I of this paper.

Major Liston has given an account of his observations on three of these forms, and since the live cultures unfortunately dried off in my hands before sub-cultures could be made, it only remains for me to add a few notes on the details of division and budding from the stained films of the larger type of amœba from liver-abscess. I have also, for the sake of completeness, given a short description of the other amœbæ, in the hope that I may be able at some future date to give the results of further work on live cultures.

### **THE LARGER TYPE OF AMŒBA FROM LIVER-ABSCESS.**

As will have been seen already from Major Liston's description, this is a very well-marked form, both from a morphological and a physiological standpoint. In stained preparations the full-grown amœba is characterised by a nucleus in which the mass of the chromatin is condensed into a large round karyosome (Pl. 16, figs. 1-5). In addition to the chromatin of the karyosome, there is a cloud of fine



granules forming a peripheral zone close beneath the nuclear membrane. With Twort's combination of neutral red and Lichtgrün (Pl. 17, figs. 17-20), or when preparations stained with iron-hæmatoxylin are counter-stained with Lichtgrün (figs. 14-16, 21), the peripheral zone is coloured green, a reaction which indicates that achromatic elements predominate in this region, and that a true achromatic membrane is present enveloping the nucleus. The dense karyosome frequently shows a central lighter region in which one or two darker grains, doubtless of the nature of centrioles, can be made out.

The numerous food-particles (chiefly ingested bacteria) which are present in the cytoplasm, and which take up the ordinary nuclear stains very strongly, render the precise study of the chromatin-grains very difficult; but in some well-stained examples there seems to be distinct evidence of passage of chromatin-granules into the cytoplasm, where they may form a more or less irregular chromidial mass surrounding the nucleus or scattered through the cytoplasm, which, consequently, is coloured a more or less deep red with the neutral red combination (Pl. 17, figs. 17-20). It will be necessary to return to this point in connection with the phenomena of budding.

As has been stated above, the large amœbæ in the liver-abscess may reproduce in the cultures in one of two ways: (1) by simple division with karyokinesis; (2) by the formation of endogenous buds.

(1) Division.—The main features of the behaviour of the nucleus in this case make it clear that the process of division is a mitosis, and this is fully confirmed by Major Liston's live observations. Unfortunately I have not been able to obtain evidence as to the method in which the nuclear spindle is formed. In the earliest examples of division that I have been able to recognise the nuclear spindle is already fully formed (Pl. 16, fig. 7), and the chromatin of the nucleus lies in the equatorial plate in the form of a number of rather irregular masses. In the later stages of division (Pl. 16,

figs. 8 and 9) the chromatin-masses have divided and separated slightly, and it is, I think, interesting to note, from a comparison of the early and late stages of division-figures (Pl. 16, fig. 7 and fig. 10), that the separation of the chromatin-masses does not appear to be due in any marked degree to the shortening of the polar threads of the spindle between the centrioles and the chromatin-masses, but rather to a growth and elongation of the separation-spindle between the two daughter-plates. In the still later stage of division shown in Pl. 16, fig. 11, the upper nucleus has practically assumed the resting condition, and would seem to show that the chromatin of the daughter-plate forms the mass of the karyosome, in which the centriole is probably included.

(2) Budding.—The most common form of reproduction for the larger type of amœbæ from liver-abscess is effected by the formation of fairly large buds, as has been described already by Major Liston in the account of his observations on the live amœbæ. There is no evidence on the stained films of this form for the formation of the numerous very small buds described by Noc (2) for a similar amœba obtained by him from liver-abscesses in Cochin-China. In the majority of the forms studied by us only one bud is formed at a time, though there are cases where this number may be exceeded (Pl. 16, fig. 4 and fig. 10).

The nucleus of the amœba takes no direct part in the formation of the bud. There is absolutely no evidence, either from observations on the live amœbæ or from the stained films, for any form of nuclear division connected with the bud-formation; and, on the other hand, it is quite clear from the stained preparations that the cytoplasm of the bud is completely cut off from that of the parent at a stage in which the bud shows no distinct nucleus. A comparison of different stages in the growth of the bud shows that the nucleus of the bud arises from chromidia contained in it when it is first formed, and derived from the chromidia scattered through the cytoplasm of the parent.

When first recognisable, the bud is seen as a small sphere,

about  $3\ \mu$  in diameter, separated from the surrounding cytoplasm of the parent by a clear space (Pl. 16, fig. 6; Pl. 17, fig. 19); at this stage the substance of the bud shows no difference from that of the parent cytoplasm, except that with Twort's stain it stains a deeper red (Pl. 17, fig. 19), indicating that the chromidia are present in greater quantity, probably as the result of growth and increase of the chromatin-substance in the bud itself after it has been cut off from the parent. The bud, imbedded completely in the maternal cytoplasm, grows until it attains a diameter of  $9-10\ \mu$  or more. The chromidia contained in the bud condense into irregular strands of chromatin (Pl. 16, figs. 2 and 3; Pl. 17, figs. 17, 18), which gradually become arranged into a nucleus of the type of that possessed by the parent (Pl. 16, fig. 4; Pl. 17, fig. 19). But by no means all the chromidia contained in the bud are used up to form its nucleus; a certain number remain over (Pl. 16, figs. 3, 4, 6; Pl. 17, figs. 18-21), so that the fully formed bud contains chromidia in its cytoplasm in addition to a nucleus. The bud at the time of its escape may show a well-developed nucleus with a karyosome (Pl. 16, fig. 4), but more frequently this change is completed after the young amœba is set free (Pl. 16, fig. 6). The young amœbæ begin to form buds in their turn long before they are full grown (Pl. 17, fig. 21).

The question of the cysts is rather a difficult one, and needs further work on live cultures. In smears from cultures about eight hours old amœbæ may be seen still enclosed in a smooth-walled cyst with the vacuole which Major Liston has described as being developed in the escaping form (Pl. 16, fig. 12). On the other hand, empty rough-walled cysts are also found. The latter may be due to a shrinkage of the cyst after the amœba has escaped, but it must be confessed that they seem rather too large to be explained on this hypothesis (Pl. 16, fig. 13).

#### THE SMALLER TYPE OF AMŒBA FROM LIVER-ABSCESS AND THE DYSENTERY-AMŒBE.

The small type of amœba from liver-abscess (Pl. 18, figs. 22-24), and the dysentery-amœbæ (Pl. 18, figs. 25-29), seem

to agree absolutely in regard to all their essential features. In both these types it seems rather difficult to stain the nucleus satisfactorily, and moreover, there seems to be evidence for a series of remarkable morphological changes in the structure of the nucleus of this form, the significance of which is not at present clear to me (Pl. 18, figs. 22-24). From the observations on the live cultures it is evident that the nuclear division is amitotic, and neither from such observations nor from the stained films is there any evidence whatever in this type for the occurrence of endogenous budding. Until more time has been spent on this form, and fresh material obtained of it, it is impossible to estimate to what extent it may differ from Schaudinn's *Entamoeba histolytica*.

As regards the other two amœbæ, that from the monkey's rectum (Pl. 18, figs. 31, 32) was on the stained films a roughly spherical form, with rather darkly staining cytoplasm, measuring about 15  $\mu$  in diameter. The cytoplasm contained numerous vacuoles. In the nucleus the chromatin was chiefly condensed in the large central karyosome, and in a distinct peripheral zone of fairly large granules. The amœba cultivated from Bombay tap-water (Pl. 18, fig. 30) measured about 16 by 10  $\mu$ ; its cytoplasm was clear, and in some cases markedly vacuolated. The nucleus showed the chromatin massed in a central karyosome.

Although I am sure that further work on these forms would lead to interesting and valuable results, yet I feel that there is already evidence amply sufficient to prove Major Liston's main contention that the larger amœba from liver-abscess is quite a distinct type, different from the other small form here described; in fact, when one regards the simple method of reproduction by endogenous buds seen in this form, it may be doubted whether it ought to be included in the amœbæ at all, but having regard to our present ignorance of this form, it may be appropriate to leave it for the present in the group which Schaudinn has pithily described as "ein Sammeltopf der heterogensten Elemente."

LISTER INSTITUTE,  
April 4th, 1911.

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## EXPLANATION OF PLATES 16–18,

Illustrating Major W. Glen Liston and C. H. Martin’s  
 “Contributions to the Study of Pathogenic Amœbæ from  
 Bombay.”

[All the figures are drawn with the camera lucida to a magnification of 2000 linear, using Zeiss objective apochr. 3 mm. homog. imm. N.A. 1.40, compens. ocular 18.]

## PLATE 16.

Large type of amœba from liver-abscess. All the preparations are stained with Heidenhain’s iron-hæmatoxylin, after fixation with Schaudinn’s fluid or sublimate-acetic.

Figs. 1–6.—Stages of endogenous bud-formation.

Figs. 1 and 2.—Early stages; in fig. 2 irregular chromidial strands are seen in the bud.

Fig. 3.—Later stage; the chromidial strands are condensing to form the nucleus of the bud.

Fig. 4.—Amœba showing six endogenous buds, one of which has the nucleus completely formed.

Fig. 5.—Extrusion of a fully-formed bud.

Fig. 6.—Free bud, with nucleus not quite fully-formed.

Figs. 7–11.—Karyokinesis and cell-division.

Fig. 7.—Early stage; the spindle is fully-formed, but the equatorial plate is not split.

Fig. 8.—Splitting of the equatorial plate.

Fig. 9.—The daughter-plates separating.

Fig. 10.—The daughter-plates widely separated; reconstitution of the daughter-nuclei beginning.

Fig. 11.—End of nuclear division; one daughter-nucleus is completely reconstituted, the other shows remains of the spindle. Division of the cell-body beginning.

Fig. 12.—Encysted amœba, about to escape from the cyst.

Fig. 13.—Empty cyst with rough wall.

#### PLATE 17.

Large type of amœba from liver-abscess. Figs. 14–16 are counter-stained with Lichtgrün-picric after iron-hæmatoxylin: figs. 17–20 are stained with Twort's combination of neutral red and Lichtgrün.

Figs. 14 and 15.—Amœbæ with ingested bacteria; in fig. 14 the karyosome appears to be breaking up.

Figs. 16–21.—Amœbæ showing endogenous bud-formation.

Fig. 16.—Amœba with two buds, one very small, in the earliest stage of formation, the other full-sized, with the chromidia beginning to form the nucleus.

Fig. 17.—Amœba with full-sized bud, which contains only scattered chromidia.

Fig. 18.—Similar stage, the nucleus of the bud beginning to be differentiated.

Fig. 19.—Amœba with three buds, two in a very early stage of formation, the third full-grown, with nucleus completely differentiated.

Fig. 20.—Amœba containing fully-formed bud.

Fig. 21.—Young amœba, not full-grown, containing a bud which is full-sized and has the nucleus in an advanced stage of differentiation.

#### PLATE 18.

Figs. 22–24.—Small type of amœbæ from the liver-abscess, showing stages of the division of the nucleus. Stained with Delafield's hæmatoxylin.

Figs. 25–29.—Amœba from dysenteric stools. Figs. 28 and 29 show early stages of nuclear division. Iron-hæmatoxylin.

Fig. 30.—Amœba isolated from Bombay tapwater. Iron-hæmatoxylin.

Figs. 31 and 32.—Amœbæ from the rectum of a monkey. In fig. 27 the nucleus has recently divided. Iron-hæmatoxylin.